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     (FILE 'HOME' ENTERED AT 16:48:29 ON 07 JUN 2004)

     FILE 'MEDLINE' ENTERED AT 16:48:43 ON 07 JUN 2004
L1           849 S SKIN (L) DERMAL (L) EPITHE?
L2           157 S L1 AND MATRIX
L3             3 S L2 AND (CELL? (L) LAMINA? (L) LAYER?)
L4             3 DUP REM L3 (0 DUPLICATES REMOVED)
L5         13390 S SKIN (L) (CONSTRUCT OR SUBSTITUTE OR GRAFT OR IMPLANT)
L6           116 S L5 AND (DERMAL (L) LAYER)
L7            49 S L6 AND FIBROBLAST

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     2004
L8           118 S L7
L9            75 DUP REM L8 (43 DUPLICATES REMOVED)
L10           62 S L9 AND PY<=2002
L11           62 FOCUS L10 1-

=> d an ti so au ab pi l11 3 5 6 7 9 11 15 21

L11  ANSWER 3 OF 62  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   1992:28231  CAPLUS
DN   116:28231
TI   Composite living skin equivalents
SO   PCT Int. Appl., 27 pp.
     CODEN: PIXXD2
IN   Eisenberg, Mark
AB   A composite living skin equivalent comprises an epidermal
     layer of cultured keratinocytes and a dermal
     layer of cultured fibroblasts in a porous crosslinked
     collagen sponge matrix.  The nonporous collagen is preferably type 1, type
     3, or a mixture thereof.  Composite grafts thus made and used in
     operations performed on children had a 90% success rate and there was no
     rejection.
     PATENT NO.        KIND  DATE           APPLICATION NO.  DATE
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PI   WO 9116010         A1   19911031       WO 1991-AU160    19910424 <--
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         RW: AT, BE, BF, BJ, CF, CG, CH, CM, DE, DK, ES, FR, GA, GB, GR, IT,
             LU, ML, MR, NL, SE, SN, TD, TG
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     CA 2080693         C    20011127
     AU 9177569         A1   19911111       AU 1991-77569    19910424 <--
     AU 632693          B2   19930107
     EP 526550          A1   19930210       EP 1991-908747   19910424 <--
     EP 526550          B1   19971229
         R:  AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE
     BR 9106354         A    19930427       BR 1991-6354     19910424 <--
     HU 63319           A2   19930830       HU 1992-3338     19910424 <--
     JP 05506169        T2   19930916       JP 1991-508090   19910424 <--
     JP 07047043        B4   19950524
     AT 161408          E    19980115       AT 1991-908747   19910424 <--
     ES 2111566         T3   19980316       ES 1991-908747   19910424 <--
     CN 1071568         A    19930505       CN 1991-109937   19911018 <--
     CN 1062441         B    20010228
     IN 174100          A    19940910       IN 1991-CA786    19911021 <--
     US 5282859         A    19940201       US 1991-777419   19911127 <--
     NO 9204034         A    19921022       NO 1992-4034     19921019 <--
     IN 177080          A    19961102       IN 1993-CA597    19931011 <--
     US 35399           E    19961210       US 1994-346525   19941129 <--

L11  ANSWER 5 OF 62  CAPLUS  COPYRIGHT 2004 ACS on STN
AN   1997:294124  CAPLUS
DN   127:9003
TI   Artificial skin composed of cultured cells and matrix
SO   Nessho (1997), 23(1), 9-27
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CODEN: NESHEG; ISSN: 0285-113X

AU Kuroyanagi, Yoshimitsu

AB A review with 220 refs. Tissue engineering is moving rapidly from the fundamental research to the com. applications. A number of cultured **skin** replacements have been produced by in vitro culture techniques. These techniques promise a new approach to the repair and reconstruction of tissues damaged by burn injury, mech. injury, and pressure sore. The 1st product, which have moved the tissue engineering potential to the com. applications, is "cultured epithelium". The pioneering work of Rheinwald and Green has demonstrated that it is possible to grow epidermal keratinocytes as stratified sheets from single cell suspension, and the resulting multilayered sheets grown in this manner have proven very effective in the management of full-thickness burns. In this regard, Compton has reported that a mature **skin** has regenerated from cultured epithelium autografts 5 yr after transplantation. Cuono has reported on an effective cultured epithelium autograft. In this method, cryopreserved allogeneic **skin** is grafted and the allogeneic epidermis is later mech. removed, and remaining allogeneic dermis is overgrafted with cultured epithelium autografts. This suggests that the **dermal** components play an important role in completing **skin** regeneration. In parallel with the acceptable concept on the need for **dermal** components, several types of bilayered **skin** replacements, consisting of both an epidermal and a **dermal** component, have been developed. This approach has been explored using the reconstructed **dermal** components, overlaid by autologous cultured keratinocytes. These **dermal** components are composed of autologous or allogeneic **fibroblasts** combined with a collagen gel or a spongy collagen-based matrix. Bell et al developed "living **skin** equivalent" which is composed of a collagen gel with **fibroblasts**, overlaid by keratinocytes. Boyce and Hansbrough developed "composite **skin substitute**" which is composed of a collagen/GAG matrix with **fibroblasts**, overlaid by keratinocytes. Kuroyanagi et al. and Maruguchi et al. also developed "composite **skin substitute**" composed of spongy collagen matrix with **fibroblasts**, overlaid by keratinocytes. These bilayered **skin** replacements are designed to function as a permanent coverage on full-thickness **skin** defects. Early surgical wound excision in patients with extensive burns has been a major advance in burn care, and rapidity of wound closure has been shown to correlate with ultimate survival of the patient. The engraftment with cadaver **skin** has been used traditionally as a "gold standard" technique. However, there are problems with supply, preservation, immune rejection, and potential infection transmission accompanying with the use of allograft **skin** . This situation underscores the need for effective alternative temporary **skin** replacements. The successful grafting of cells across major histocompatibility barriers suggests that grafted cells are either nonimmunogenic or so weakly immunogenic that immunol. rejection could not be detected clin. Keratinocytes and **fibroblasts** do not constitutively express class II antigens. These cells may lack the antigenicity necessary to elicit an immune response. They would therefore be feasible for allograft use. On the basis of this concept, allogeneic "cultured **dermal** replacement" has been developed. Hansbrough et al. developed 2 types of "living **skin** replacement". One is composed of **fibroblasts** grown on the nylon mesh surface of Biobrane®, a synthetic wound dressing, consisting of silicone membrane bonded to one surface of the nylon mesh. Another is composed of **fibroblasts** grown on the synthetic biodegradable matrix, polyglactin mesh. Kuroyanagi developed "cultured **dermal** replacement" in which **fibroblasts** cultured on the spongy collagen matrix. Allogeneic cultured epithelium, prepared by the technique of Rheinwald and Green, has proven very effective in the management of split-thickness **skin** defects. Hansbrough et al. developed "cultured epidermal replacement" in which keratinocytes cultured to single-**layer** confluence on Hydroderm®, a synthetic wound dressing, consisting of hydrophilic polyurethane membrane. These allogenic living **skin** replacements, i.e., cultured **dermal** and epidermal replacements, are expected to be more widely used. These function as "biol. wound dressing", since incorporated cells

are able to release biol. active substances such as cytokines.

L11 ANSWER 6 OF 62 MEDLINE on STN
AN 94064754 MEDLINE
TI Composite **grafts** of human keratinocytes grown on a polyglactin mesh-cultured **fibroblast** dermal **substitute** function as a bilayer **skin** replacement in full-thickness wounds on athymic mice.
SO Journal of burn care & rehabilitation, **(1993 Sep-Oct)** 14 (5) 485-94.
Journal code: 8110188. ISSN: 0273-8481.
AU Hansbrough J F; Morgan J L; Greenleaf G E; Bartel R
AB We have developed and tested in athymic mice a new, cultured, **dermal**-epidermal **graft** composed of two human cell types coupled with a biodegradable **dermal** scaffold. Cultured, proliferating human keratinocytes (HK) were applied to the surface of a living **dermal** tissue replacement that is composed of human **fibroblasts** cultured on a polyglactin mesh. After 4 to 6 days of coculture, proliferating HKs achieved confluency on the surface of the living **dermal** tissue replacement. **Grafts** were then transferred to full-thickness wounds on the dorsum of athymic mice. Sixteen animals were grafted, and the mean percentage of **graft** take (original wound area covered) on day 20 after grafting was 51.25%. Staining with antibody specific for human involucrin confirmed the presence of HKs on closed wounds, and staining with antibody specific for human laminin revealed a continuous **layer** of laminin at the **dermal**-epidermal junction on day 20. Animals closed with living **dermal** tissue replacement alone markedly contracted, whereas application of living **dermal** tissue replacement-HK **grafts** appeared to retard contraction. Because polyglactin mesh fibers are absorbed by hydrolysis rather than by enzymatic degradation, this living composite **graft** may be more resistant to destruction when placed on excised human wounds than are composite **grafts**, which are composed of a collagen matrix. The inclusion of the living **dermal substitute** may ultimately provide better **skin** quality than is achieved from the use of cultured keratinocytes alone. Fragility of the epidermal **layer** is probably due to the short-term culture of HKs on the living **dermal** tissue replacement, and further efforts to develop a thicker epithelial **layer** may improve **graft** durability.

L11 ANSWER 7 OF 62 MEDLINE on STN
AN 92213592 MEDLINE
TI Evaluation of a biodegradable matrix containing cultured human **fibroblasts** as a dermal replacement beneath meshed **skin grafts** on athymic mice.
SO Surgery, **(1992 Apr)** 111 (4) 438-46.
Journal code: 0417347. ISSN: 0039-6060.
AU Hansbrough J F; Cooper M L; Cohen R; Spielvogel R; Greenleaf G; Bartel R L; Naughton G
AB Meshed, expanded split-thickness **skin grafts** (MSTSG) frequently achieve poor results when used to cover full-thickness wounds. Poor cosmetic and functional results occur in part because the epithelium that grows across the **skin graft** interstices lacks a dermis. We used a living **dermal** replacement composed of either polyglycolic acid (PGA) or polyglactin-910 (PGL) mesh containing confluent, cultured human **fibroblasts**. These **grafts** were applied to full-thickness wounds on athymic mice; widely expanded, 3:1 ratio human MSTSG was then placed over the **dermal graft**. Histologic examination of wounds during a 99-day period after **graft** placement showed that PGA/PGL-**fibroblast grafts** vascularized to the wound, and the MSTSG simultaneously vascularized to the PGA/PGL-**fibroblast graft**. Epithelialization from the MSTSG bridges proceeded rapidly across the surface of the PGA/PGL-**fibroblast grafts**, resulting in an epithelialized **layer** that covered a densely cellular substratum that resembled dermis. Basement membrane formation at the **dermal**-epidermal junction of the epithelialized interstices was confirmed by immunohistochemical microscopy. Minimal inflammatory

reaction to the PGA/PGL-**fibroblast grafts** was seen. **Grafts** composed of PGA or PGL biodegradable meshes combined with cultured **fibroblasts** vascularize in full-thickness wounds, resulting in formation of organized tissue beneath the epithelialized surface that resembles dermis.

L11 ANSWER 9 OF 62 MEDLINE on STN
AN 90300729 MEDLINE
TI In vitro effects of matrix peptides on a cultured dermal-epidermal **skin substitute**.
SO Journal of surgical research, **(1990 Jun)** 48 (6) 528-33.
Journal code: 0376340. ISSN: 0022-4804.
AU Cooper M L; Hansbrough J F; Foreman T J
AB Composite **dermal**-epidermal **skin substitutes** rely on a firm attachment of human keratinocytes (HK) to the **dermal** substrate for **graft** survival on the wound. An in vitro study was performed assessing whether the addition of matrix peptides to the **dermal** substrate effected the epithelial thickness. Cultured **grafts** were made by attaching HK to the external surface of a collagen-chondroitin 6-sulfate (GAG) membrane and inoculating human **fibroblasts** (HF) internally. If the matrix peptide (RGD Peptide) was added to the collagen-GAG membrane prior to placement of the HK and HF, the resultant epithelial **layer** at the end of the normal 4-day culture period was significantly thicker (19.7 +/- 0.9 microns versus 13.5 +/- 1.0 microns). Subjectively, the HF content was also greater on the peptide-treated **grafts**. When HF were not placed on the cultured **graft**, i.e., only collagen-GAG membrane, RGD peptide, and HK, the resultant epithelial thickness was even greater (28.3 +/- 1.0 microns). These data suggest that addition of matrix peptides, which increase cell attachment to the **dermal** substrate, may prove effective in the improvement of this cultured composite **dermal**-epidermal **skin substitute**.

L11 ANSWER 11 OF 62 MEDLINE on STN
AN 2000126510 MEDLINE
TI Cellular artificial **skin substitute** produced by short period simultaneous culture of **fibroblasts** and keratinocytes.
SO British journal of plastic surgery, **(1999 Oct)** 52 (7) 573-8.
Journal code: 2984714R. ISSN: 0007-1226.
AU Kim B M; Suzuki S; Nishimura Y; Um S C; Morota K; Maruguchi T; Ikada Y
AB We developed a novel artificial **skin substitute** consisting of two collagen sponge **layers** with different pore sizes and cross-link densities. **Fibroblasts** suspended in 0.5 ml Dulbecco-modified Eagle's medium (DMEM) + 10% fetal bovine serum (FBS) were seeded on the lower **dermal** sponge **layer**, then epidermal collagen sponge and 0.1 ml suspension of keratinocytes in KGM were layered in this order. After a few hours, the medium was changed to DMEM + 5% FBS. These processes were carried out in one day, and the composite **layers** were then cultured by the air-liquid interface culture method. Three to five days after seeding, keratinocytes had grown to about ten **layers**, and **fibroblasts** had grown three-dimensionally into the lower **dermal** sponge **layer** . This novel cellular artificial **skin substitute** was grafted onto nude mice and took in 4 weeks. This **skin substitute** has the advantage of a shorter culturing period than previously cultured **skins**, and may be clinically useful for grafting that is urgently required in patients with severe generalised burns.

L11 ANSWER 15 OF 62 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
AN 94:203830 SCISEARCH
TI DEVELOPMENT OF A BILAYERED LIVING **SKIN CONSTRUCT** FOR CLINICAL-APPLICATIONS
SO BIOTECHNOLOGY AND BIOENGINEERING, (**05 APR 1994**) Vol. 43, No. 8, pp. 747-756.
ISSN: 0006-3592.
AU WILKINS L M; WATSON S R; PROSKY S J; MEUNIER S F; PARENTEAU N L (Reprint)
AB An in vitro **construct** of human **skin** (living

skin equivalent, LSE) has been engineered using serially passaged human epidermal keratinocytes and human **dermal fibroblasts** with a matrix of type I collagen. Cells are obtained from neonatal foreskin. LSE is cast, cultured, and shipped in a single culture insert. The size and shape of the insert determines the size and shape of the LSE. The **dermal** matrix consists of **dermal fibroblasts** within a condensed collagen lattice. The overlying epidermis is developed at the air-liquid interface to generate a protective cornified **layer**. Serum was not necessary for development of the epidermis. LSE for **graft** (Graftskin) has handling characteristics similar to split-thickness **skin** allowing it to be meshed, stapled, and sutured. LSE was cryopreserved using 65% glycerol and rapid freezing. Viability and in vivo performance on athymic mice were similar to fresh LSE. Cells derived from human eccrine gland were able to invade and form tubules within the **dermal** matrix indicating that the addition of rudimentary appendages may be possible. (C) 1994 John Wiley and Sons, Inc.

L11 ANSWER 21 OF 62 MEDLINE on STN
AN 2001462033 MEDLINE
TI In vitro characterization of an artificial dermal scaffold.
SO Tissue engineering, **(2001 Aug)** 7 (4) 457-72.
Journal code: 9505538. ISSN: 1076-3279.
AU Ojeh N O; Frame J D; Navsaria H A
AB The treatment of extensive burn injuries has been enhanced by the development of artificial **skin substitutes**. Integra Artificial **Skin**, an acellular collagen-glycosaminoglycan (C-GAG) **dermal** equivalent requires a two-stage grafting procedure. However, preseeding the C-GAG **dermal** equivalent with cultured **fibroblasts** and keratinocytes, with the aim of performing a single-stage grafting procedure, may be beneficial in terms of replacing the requirement for traditional split-**skin grafts**. In this comparative in vitro study, the interactions of cultured human **dermal fibroblasts** and epidermal keratinocytes in Integra Artificial **Skin** in comparison to cadaver deepidermalized dermis (DED) was investigated. An increase in cell proliferation and migration in the C-GAG **dermal** equivalent was observed over time. Cocultures of **fibroblasts** and keratinocytes on both **dermal** equivalents showed positive expression of proliferation, differentiation, and extracellular matrix (ECM) protein markers. Organization of keratinocytes in the epidermal **layers** of DED composites were better compared to the C-GAG composites. Deposition of ECM proteins was enhanced in the presence of keratinocytes in both **dermal** equivalents. Results demonstrate that in vitro the C-GAG **dermal** equivalent is biocompatible for cell attachment, migration, proliferation, and differentiation. Preseeding Integra Artificial **Skin** with cultured autologous **fibroblasts** and keratinocytes for in vivo application, as a single-stage grafting procedure, warrants testing. A better clinical outcome may be achieved as shown by our in vitro results of the coculture composites.

L Number	Hits	Search Text	DB	Time stamp
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